

FORM-PTO-1390 (Modified) (REV 7-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER <b>21508-033</b>
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR) <b>09/937735</b>
INTERNATIONAL APPLICATION NO. <b>PCT/US99/07745</b>	INTERNATIONAL FILING DATE <b>08 April 1999 (08.04.99)</b>	PRIORITY DATE CLAIMED	
TITLE OF INVENTION <b>INDUCTION OF KIDNEY TUBULE FORMATION</b>			
APPLICANT(S) FOR DO/EO/US <b>MCMAHON, Andrew P.; KISPERT, Andreas; VAINIO, Seppo.</b>			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.</li> <li>4. <input checked="" type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31).</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2))           <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input type="checkbox"/> has been communicated by the International Bureau</li> <li>c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).           <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> is attached hereto.</li> <li>b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4)</li> </ol> </li> <li>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))           <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input type="checkbox"/> have been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input checked="" type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).</li> <li>10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5))</li> <li>11. <input checked="" type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409).</li> <li>12. <input checked="" type="checkbox"/> A copy of the International Search Report (PCT/ISA/210)</li> </ol>			
<b>Items 13 to 20 below concern document(s) or information included:</b>			
<ol style="list-style-type: none"> <li>13. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li>14. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li>15. <input type="checkbox"/> A <b>FIRST</b> preliminary amendment.</li> <li>16. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li> <li>17. <input type="checkbox"/> A substitute specification.</li> <li>18. <input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li>19. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.</li> <li>20. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4).</li> <li>21. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).</li> <li>22. <input checked="" type="checkbox"/> Certificate of Mailing by Express Mail</li> <li>23. <input checked="" type="checkbox"/> Other items or information:</li> </ol>			
<b>Express Mail Label No: EL390884359US</b> <b>Filed On: 28 September 2001 (28.09.01)</b>			


JP12 Rec'd PCT/PTO 28 SEP 2001

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.53) <b>09/937735</b>	INTERNATIONAL APPLICATION NO. <b>PCT/US99/07745</b>	ATTORNEY'S DOCKET NUMBER <b>21508-033</b>
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24. The following fees are submitted: <b>BASIC NATIONAL FEE ( 37 CFR 1.492 (a) (1) - (5) ) :</b> <input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... <b>\$1000.00</b> <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... <b>\$860.00</b> <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... <b>\$710.00</b> <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... <b>\$690.00</b> <input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... <b>\$100.00</b> <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b> <b>\$100.00</b>				<b>CALCULATIONS PTO USE ONLY</b>	
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).				<b>\$130.00</b>	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	21 - 20 =	1	x \$18.00	<b>\$18.00</b>	
Independent claims	3 - 3 =	0	x \$80.00	<b>\$0.00</b>	
Multiple Dependent Claims (check if applicable). <input type="checkbox"/>				<b>\$0.00</b>	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				<b>\$248.00</b>	
<input type="checkbox"/> Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				<b>\$0.00</b>	
<b>SUBTOTAL =</b>				<b>\$248.00</b>	
Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).				<b>\$0.00</b>	
<b>TOTAL NATIONAL FEE =</b>				<b>\$248.00</b>	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). <input type="checkbox"/>				<b>\$0.00</b>	
<b>TOTAL FEES ENCLOSED =</b>				<b>\$248.00</b>	
				<b>Amount to be: refunded</b>	<b>\$</b>
				<b>charged</b>	<b>\$</b>

- a. ☒ A check in the amount of **\$248.00** to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \_\_\_\_\_ to cover the above fees. A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **50-0311** A duplicate copy of this sheet is enclosed.
- d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO: <b>BEATTIE, Ingrid A.</b> <b>Mintz, Levin, Cohn, Ferris, Glovsky &amp; Popeo, P.C.</b> <b>One Financial Center</b> <b>Boston, Massachusetts 02111</b> <b>United States of America</b>	 SIGNATURE <b>Ingrid A. Beattie</b> NAME <b>42,306</b> REGISTRATION NUMBER <b>28 September 2001 (28.09.01)</b> DATE
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Attorney Docket No. 21508-033NatI

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

APPLICANTS : McMahon *et al.*  
SERIAL NUMBER : 09/937,735 EXAMINER : Not Yet Assigned  
FILING DATE : Sept 28, 2001 ART UNIT : Not Yet Assigned  
FOR : INDUCTION OF KIDNEY TUBULE FORMATION

April 29, 2002  
Boston, Massachusetts

U.S. Patent and Trademark Office  
BOX Sequence  
P.O. 2327  
Arlington, VA 22202

**PRELIMINARY AMENDMENT**

Prior to examination of the above-identified patent application, please amend the application as set forth below and consider the following remarks.

In the Specification:

Please insert the Sequence Listing, pages 1-10, at the end of the specification.

**REMARKS**

Applicants submit a Sequence Listing for the nucleotide sequences disclosed in the specification, in compliance with the requirements for patent applications containing nucleotide sequences and/or amino acid sequence disclosures. 37 C.F.R. §§ 1.821-1.825.

**CONCLUSION**

Applicants respectfully submit that the present application complies with 37 C.F.R. §§ 1.821-1.825. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,



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INDUCTION OF KIDNEY TUBULE FORMATION

Statement as to Federally Sponsored Research

5        This invention was funded in part by the  
 U.S. Government under grant number HD30249 awarded by the  
 National Institutes of Health. The Government has  
 certain rights in the invention.

Background of the Invention

10        Kidney and urinary tract diseases are major causes  
 of illness and death in the United States resulting in  
 about 50,000 deaths per year. Renal cell carcinoma is  
 the most common type of kidney cancer; this type of  
 cancer affects the lining of the renal tubule and is  
 15 often metastatic. About one third of the cases diagnosed  
 show metastasis, e.g., to the lung or other organs, at  
 the time of diagnosis. Other types of medical  
 conditions, such as diabetes mellitus and high blood  
 pressure, can lead to chronic kidney failure.  
 20 Current therapeutic approaches include dialysis and  
 transplantation.

Summary of the Invention

The invention provides a method of regenerating  
 kidney tissue and is based on the discovery that Wnt-4 is  
 25 sufficient to trigger kidney tubulogenesis, whereas  
 Wnt-11 (which is also involved in tubule formation) is  
 not. Kidney tubule formation in a post-natal mammal is  
 stimulated by administering to the mammal a substantially  
 pure Wnt polypeptide or a Wnt agonist. Preferably, the  
 30 Wnt polypeptide is Wnt-4 or a Wnt-1 class polypeptide  
 such as Wnt-1, Wnt-2, Wnt-3a, Wnt-7a, and Wnt-7b. A Wnt-  
 1 class polypeptide is a Wnt polypeptide that transforms  
 C57MG cells in culture. More preferably, the polypeptide  
 is Wnt-3a, Wnt-4, Wnt-7a, and Wnt-7b, but not members of  
 35 the Wnt-5a class of proteins such as Wnt-5a or Wnt-11.  
 For example, the Wnt polypeptide is Wnt-4, and the Wnt

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agonist is HLDAT86. Wnt-4 mediated-tubulogenesis requires cell contact; accordingly, Wnt compositions are preferably administered to kidney cells in the context of the kidney organ or in a situation in which the cells  
5 expressing a Wnt polypeptide or agonist are in close contact with cells involved in tubule formation. In preferred embodiments, sulfated glycosaminoglycans (sGAGs) are co-administered with the Wnt compositions.

The mammal to be treated is characterized as  
10 suffering from a kidney disorder. Preferably, the mammal is a human, mouse, rat, guinea pig, cow, sheep, horse, pig, rabbit, monkey, dog, or cat. The method is therapeutic or preventative and is administered to a juvenile or adult mammal. Kidney disorders include  
15 chronic renal failure, renal cell carcinoma, polycystic kidney disease, chronic obstructive uropathy, and virus-induced nephropathy. For example, the method is used to treat or prevent renal tubule epithelial cell degeneration associated with HIV-1 infection.

20 Administration of the Wnt compositions is local or systemic. For example, the polypeptide or Wnt agonist is administered locally to a renal tissue by, e.g., retrograde perfusion of renal tissue via blood vessels or urine collecting ducts. Wnt compositions are also  
25 administered ex vivo to an explanted renal tissue. For example, a kidney is removed from an individual and treated *in vitro* with a Wnt composition (e.g, a substantially pure polypeptide or an isolated nucleic acid) and then returned to the body of the same  
30 individual or a different individual.

The Wnt composition is a peptide mimetic, e.g., a polypeptide that is more resistant to proteolytic cleavage compared to a naturally-occurring Wnt polypeptide. The Wnt polypeptide is preferably soluble  
35 under physiological conditions. Accordingly, the

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polypeptide is modified to improve its solubility. Alternatively, the Wnt polypeptide is present on the surface of a cell. The method utilizes a Wnt polypeptide that includes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO:1, 2, 3, 4, or 5, a Wnt polypeptide that includes an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO:1, 2, 3, 4, or 5, a Wnt polypeptide that includes an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:1, 2, 3, 4, or 5, and a Wnt polypeptide that includes an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO:1, 2, 3, 4, or 5. The Wnt polypeptide preferably has an amino acid sequence at least 85% identical to SEQ ID NO: and functions to stimulate tubulogenesis. For example, the polypeptide may be a fragment of Wnt that stimulates tubulogenesis. A fragment has an amino acid sequence that is identical to part, but not all, of the amino acid sequence of a naturally-occurring Wnt polypeptide. A fragment of a naturally-occurring Wnt polypeptide that stimulates tubulogenesis preferably includes the amino acid sequence of at least the amino-terminal 50% of the naturally-occurring polypeptide. More preferably, the fragment contains the amino acid sequence of at least the amino terminal 75% of a naturally-occurring Wnt polypeptide. For example, the fragment contains at least residues 1-180 of naturally-occurring Wnt-1 (SEQ ID NO:1). Other fragments of Wnt polypeptides which have been shown to stimulate tubulogenesis, e.g., residues 100-331 of naturally-occurring Wnt-7a (SEQ ID NO:4, highlighted in bold), are administered. Full-length Wnt polypeptides or fragments thereof are chemically or recombinantly linked to Ig to yield Wnt-Ig fusion proteins. Human or mouse Wnt polypeptides are administered to mammals to stimulate

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tubulogenesis.

Also within the invention is a method of stimulating kidney tubule formation in a post-natal mammal by administering a substantially pure or isolated nucleic acid encoding a Wnt polypeptide (e.g., a nucleic acid having the nucleotide sequence of SEQ ID NO:10, 11, or 12) or a Wnt agonist. Nucleic acids that encode a Wnt polypeptide and that have a sequence that is substantially identical to a Wnt-encoding nucleic acid sequence are administered to diseased kidney tissue.

Polypeptides or other compounds of interest are said to be "substantially pure" when they are within preparations that are at least 60% by weight (dry weight) the compound of interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

A polypeptide or nucleic acid molecule which is "substantially identical" to a given reference polypeptide or nucleic acid molecule is a polypeptide or nucleic acid molecule having a sequence that has at least 85%, preferably 90%, and more preferably 95%, 98%, 99% or more identity to the sequence of the given reference polypeptide sequence or nucleic acid molecule.

"Identity" has an art-recognized meaning and is calculated using well known published techniques, e.g., Computational Molecular Biology, 1988, Lesk A.M., ed., Oxford University Press, New York; Biocomputing: Informatics and Genome Projects, 1993, Smith, D.W., ed., Academic Press, New York; Computer Analysis of Sequence Data, Part I, 1994, Griffin, A.M. and Griffin, H.G., eds, Humana Press, New Jersey; Sequence Analysis in Molecular Biology, 1987, Heinje, G., Academic Press, New York; and

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Sequence Analysis Primer, 1991, Gribskov, M. and Devereux, J., eds., Stockton Press, New York). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans and has a definite meaning with respect to a given specified method. Sequence identity is measured using the Sequence Analysis Software Package of the Genetics Computer Group (GCS), University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705), with the default parameters as specified therein.

By "isolated nucleic acid molecule" is meant a nucleic acid molecule that is free of the genes which, in the naturally-occurring genome of the organism, flank a gene encoding a Wnt polypeptide. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence such as an immunoglobulin polypeptide. The term excludes large segments of genomic DNA, e.g., such as those present in cosmid clones, which contain a gene of interest flanked by one or more other genes which naturally flank it in a naturally-occurring genome.

30 Nucleic acid molecules include both RNA and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. Where single-stranded, the nucleic acid molecule may be a sense strand or an antisense strand. The term therefore includes, for 35 example, a recombinant DNA which is incorporated into a

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vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote at a site other than its natural site; or which exists as a separate molecule (e.g., a cDNA or a genomic  
5 or cDNA fragment produced by polymerase chain reaction (PCR) or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence such as an Ig polypeptide.

10 Wnt nucleic acids (encoding Wnt polypeptides) which hybridize at high stringency to naturally-occurring Wnt-encoding sequences are also administered to stimulate tubulogenesis. Hybridization is carried out using standard techniques such as those described in Ausubel et  
15 al., *Current Protocols in Molecular Biology*, John Wiley & Sons, (1989). "High stringency" refers to DNA hybridization and wash conditions characterized by high temperature and low salt concentration, e.g., wash conditions of 65° C at a salt concentration of  
20 approximately 0.1 X SSC. "Low" to "moderate" stringency refers to DNA hybridization and wash conditions characterized by low temperature and high salt concentration, e.g. wash conditions of less than 60° C at a salt concentration of at least 1.0 X SSC. For example,  
25 high stringency conditions may include hybridization at about 42°C, and about 50% formamide; a first wash at about 65°C, about 2X SSC, and 1% SDS; followed by a second wash at about 65°C and about 0.1% x SSC. Lower stringency conditions suitable for detecting DNA  
30 sequences having about 50% sequence identity to *csa-1* gene are detected by, for example, hybridization at about 42°C in the absence of formamide; a first wash at about 42°C, about 6X SSC, and about 1% SDS; and a second wash at about 50°C, about 6X SSC, and about 1% SDS.

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The invention also includes an *ex vivo* mammalian kidney containing an exogenous Wnt polypeptide, e.g., having been bathed in or perfused with a solution containing a Wnt polypeptide or agonist. Alternatively, the *ex vivo* mammalian kidney contains exogenous DNA encoding a Wnt polypeptide. The kidney is bathed or perfused with a solution containing a Wnt-encoding nucleic acid, and cells of the kidney take up the DNA. The cells then express and secrete the recombinant Wnt polypeptide or agonist. For expression of recombinant Wnt polypeptides, Wnt-encoding sequences are operably linked to regulatory sequences, e.g., tissue specific promoters. Kidney-specific promoters are known in the art and include, e.g., the Pax-2 promoter, the cRET promoter, and the Hox b7 promoter. By "operably linked" is meant able to promote transcription of an mRNA corresponding to a polypeptide-encoding sequence located downstream on the same DNA strand.

#### Description of the Preferred Embodiments

A Wnt polypeptide, e.g., Wnt-4, Wnt-1, Wnt-3a, Wnt-7a and Wnt-7b, acts as a trigger to start an intrinsic program in the mesenchymal cells which then proceed to form complex nephron like structures. Wnt-4 is a secreted glycoprotein which is required for kidney tubule formation. Development of the mammalian kidney is initiated by ingrowth of the ureteric bud into the metanephric blastema. In response to signal(s) from the ureter, mesenchymal cells condense, aggregate into pretubular clusters, and undergo epithelialisation to form simple epithelial tubules. Subsequent morphogenesis and differentiation of the tubular epithelium lead to the establishment of a functional nephron.

#### Table 6: Human Wnt-1 amino acid sequence

1 MGLWALLPGW VSATLLLLALA ALPAALAANS SGRWWGIVNV ASSTNLLTDS  
35 KSLQLVLEPS

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61 LQLLSRKQRR LIRQNPGILH SVSGGLQSAV RECKWQFRNR RWNCPATGPG  
HLFGKIVNRG  
121 CRETAFIFAI TSAGVTHSVA RSCSEGSIES CTCDYRRRGPP GGPDWHWGGC  
SDNIDFGRLF  
5 181 GREFVDSGEK GRDLRFLMNL HNNEAGRRTTV FSEMRQECKC HGMSGSCCTVR  
TCWMRLPTLR  
241 AVGDVLRDRF DGASRVLYGN RGSNRASRAE LLRLEPEDPA HKPPSPHDLV  
YFEKSPNFCT  
301 YSGRLGTAGT AGRACNSSSP ALDGCELLCC GRGHRTRTQR VTERCNCTFH  
10 WCCHVSCRNC  
361 THTRVLHECL (SEQ ID NO:1)

Table 7: Human Wnt-3a amino acid sequence

CKCHGLSGSC EVKTCWWSQP DFRAIGDFLK DKYDSASEMV VEKHRESRGW  
VETLRPRYTY FKVPTERDLV YYEASPNFCE PNPETGSFGT RDRTCNVSSH  
15 GIDGCDLLCC GRGHNARAER RREKCRCVFH WCC (SEQ ID NO:2)

Table 8: Human Wnt-4 amino acid sequence

CKCH GVSQSCEVKT CWRAVPPFRQ VGHALKEKFD GATEVEPRRV GSSRALVPRN AQFKPHTDED  
LVYLEPSPDF CEQDMRSGVL GTRGRTCNTK SKAIDGCELL CCGRGFHTAQ  
VELAERCSCCK  
20 FHWCLFLSR (SEQ ID NO:3)

Table 9: Human Wnt-7a amino acid sequence

1 MNRKALRCLG HLFLSLGMVC LRIGGFSSVV ALGATIICNK IPGLAPRQRA ICQSRPDAII  
61 VIGEGSQMGL DECQFQFRNG RWNCALGER TVFGKELKVG SRDGAFTYAI IAAGVAHAIT  
121 AACTHGNLSD CGCDKEKQGG YHRDEGWKVG GCSADIRYGI GFAKVVDAR EIKQNARTLM  
25 181 NLHNNEAGRK ILEENMKLEC KCHGVSGSCT TKTCWTTLPQ FRELGYVLKD KYNEAVHVEP  
241 VRASRNKRPT FLKIKKPLSY RKPMDTDLVY IEKSPNYCEE DPVTGSGVTQ GRACNKTAPQ  
301 ASGCDLMCCG RGYNTHQYAR VWQCNCCKFHW CCYVKCNTCS ERTEMYTCK  
(SEQ ID NO:4)

Table 10: Human Wnt-7b partial amino acid sequence

30 VKC GVSQSCTTKT CWTTLPKFRE VGHLLKEKYN AAVQVEVVRA SRLRQPTFLR IKQLRSYQKP  
METDLVYIEK SPNYCEEDAA TGSVGTQGR I CNRTSPGADG CDTMCCGRGY NTHQYTKVWQ  
CNCKFHWCCS (SEQ ID NO:5)

Table 11: Human Wnt-1 Nucleotide Sequence

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1 atgtatgtat gtatgtatgt atgtatgtat acgtgctgtc acctgtgtgt
gcttgggtgtc
61 agtgggggtc agacatcacc tgattccctg gaactggagt tacagggtggc
5 tataagccac
121 cacttgggtg ctgagaacag agtccgggcc tctggcagag cagtcagtgc
ttttagccac
181 tgagccactc tcatcccccc aattatgttc atcttgagtt gggcaggtac
ggtggcggaa
10 241 taggcctgta atcccagcag tcaactggacc atcatgggtt ctacatatta
aaccttttatg
301 ttaggtaggg tcacacagca agatccggtc acaaaaccag caacaacaaa
aaccaaaagg
361 agccagcttc ttcccacaag cattctttcc ctcaggtctt cagctccatc
15 tgacagctac
421 tcggctgggtg gtcctatcct ttctgagcct agttgccaga gaaacaagcc
cggttcatct
481 tcatgactag cacatctaata gataagcaca ggttgactca aggtgccata
gagtgacact
20 541 aggtaccacag agcgacagaa tgacacctat gagtgcacgt cgttaatcac
aaacacacac
601 acacacacac acacacacac acacacacac tcatgcaccc acctgcaaac
acaattgcag
661 ccttctggac gtctcctgtc acagccccac ctcttctctg atacactgcg
25 ttaagtgggtg
721 actgtaacaa aatgacttca tgctctccct gtctgagcc aaattacaca
attatttggga
781 aagggtcaa aatgttcttc gttagaagtt tctggataca ccaatacaca
ggagcgtgca
30 841 ccctcagaac acatgtacac tttgacttaa tctcacgggt gacacaccga
cgcttacact
901 cccctagacc cacagaggca aactgctggg cgcttctgag tttctcactg
ccaccagctc
961 ggtttgctca gcctaccccc gcaccccgcg cccgggaatc cctgaccaca
35 gctccacca
1021 tgctctgtct ccttcttttc cttctctgtc cagccgtcgg ggttcctggg
tgaggaagtg
1081 tctccacgga gtcgctggct agaaccacaa ctttcatcct gccattcaga
atagggaaga
40 1141 gaagagacca cagcgtaggg gggacagagg agacggactt cgagaggaca
gccccaccgg
1201 cgcgtgtggg ggaggcaatc caggctgcaa acaggttgct cccagcgcat
tgtccccgcg

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	1261	ccccctggcg	gatgctggtc	cccgaacggc	tccggacgcg	cagaagagt	g
		agggcggcgc					
	1321	gcgtgggagg	ccatcccaag	gggaggggtc	ggcggccagt	gcagacctgg	
		agggcggggcc					
5	1381	accaggcagg	gggcgggggt	gagccccgac	ggttagcctg	tcagctcttt	
		gctcagaccg					
	1441	gcaagagcca	cagcttcgct	cgccactcat	tgtctgtggc	cctgaccagt	
		gcgccctggg					
	1501	gcttttagtg	ccgcccgggc	ccggaggggc	agcctcttct	cactgcagtc	
10		agcgccgcaa					
	1561	ctataagagg	cctataagag	gcggtgcctc	ccgcagtggc	tgcttcagcc	
		cagcagccag					
	1621	gacagcgaac	catgctgcct	gcggccccgc	tccagactta	ttagagccag	
		cctgggaact					
15	1681	cgcatactg	ccctcaccgc	tgtgtccagt	cccaccgtcg	cggacagcaa	
		ccacagtcgt					
	1741	cagaaccgca	gcacagaacc	agcaaggcca	ggcaggccat	ggggctctgg	
		gcgctgctgc					
	1801	ccagctgggt	ttctactacg	ttgctactgg	cactgaccgc	tctgcccgca	
20		gccctggctg					
	1861	ccaacagtag	tggccgatgg	tggtaaagtga	gctagtacgg	ggtccgccac	
		ttgtcctggg					
	1921	gcaaagagcc	aggcacgggc	cttaccacgc	tcccacgctg	tggggatcac	
		caacctacag					
25	1981	acccccctcg	tgcattgtga	cttcacatcc	agggtgctca	cacctagaac	
		tagctctgct					
	2041	gaagtggggc	acatacttgg	catgcagaag	cccagataca	ccaggctcag	
		agaccattcc					
	2101	catttaatac	gaccccgttt	ctgctgagca	acaggtccca	acctcgctgt	
30		ggtgggtgct					
	2161	caggtgtccc	ttagggtcttg	aacccaaaaa	aaaaaaaaaa	aaaaaaaaaa	
		accagatatt					
	2221	agctttgagg	tgagggagtg	gaattcctaa	gtttttcaag	gtgggcaagg	
		ctgcagggtg					
35	2281	ggtttctcct	cgggggctga	cttgaagaaa	ggaagagcta	aggtagccat	
		gccttttctg					
	2341	tccactcact	agactctgga	gctcagggcc	aggcaaggat	agggtggtac	
		agcctgtatg					
	2401	gttaggatgc	agggtcccctc	ccctggactg	aacccttatg	catcccgcc	
40		ggggcatcgt					
	2461	gaacatagcc	tctccacga	acctgttgac	ggattccaag	agtctgcagc	
		tggtgctcga					
	2521	gccagctctg	cagctgctga	gccgcaagca	gcggcgactg	atccgacaga	
		acccqqqqat					

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2581 cctgcacagc gtgagtggag ggctccagag cgctgtgcga gagtgcacaa  
ggcaattccg  
2641 aaaccgccgc tggaactgcc cactgctcc ggggccccac ctcttcggca  
agatcgtaa  
5 2701 ccgaggtggg tgcccaggaa agcgacgctt ccgggattaa gggaaaagca  
gggtcatctc  
2761 cagggcatag gcgggcgaag gcaggaaga catcccaggg ttatatgtga  
tcaaactgag  
2821 aatcgccctg tgccggcagt taccgtaggc cagcaccaga ttctttctag  
10 ccttgcggtg  
2881 tgagcatgat ctttaacgtt gctggccact ggcccacaga aagggaaattc  
cggatcgagg  
2941 gcgctgggag acagctgttt ttccctagcc ttctcaaag gtacctggga  
agctgatctc  
15 3001 tgagggctag ctaggggtgt gcttcgacc cagcaaagt tgactgcca  
atactagtag  
3061 cgatcttggc tatgcagatt tgttctactt gggaatctcc ccttggaagt  
gctctgctag  
3121 ggctctggag tctcagtaaa gcttagagag gagggcattc catgcttcgc  
20 acacatgact  
3181 ccaaggatgt tggactgtag ggtaccaagt ctcccaaaca ggggtgctgag  
ttggccccac  
3241 gccttctctc aactgatgcg gggtcgcttc acccacaggc tgccgagaaa  
cagcgttcat  
25 3301 cttegcaatc acctccgccg gggtcacaca ttccgtggcg cgctcctgct  
ccgaaggctc  
3361 catcgagtcc tgcacctgcg actaccggcg gcgcggccct gggggccccg  
actggcactg  
3421 ggggggctgc agtgacaaca tcgattttgg tcgcctcttt ggccgagagt  
30 tcgtggactc  
3481 cgggggagaag gggcgggacc tacgcttctt catgaacctt cacaacaacg  
aggcagggcg  
3541 aacggtacgt cgggtgtgtc ggaaccaatg gcaggggaga tgtaagacag  
gtgcacgggg  
35 3601 acagaggcac agggaggggc ttcccagag agtgggactc taggagggaa  
gacagagaag  
3661 aggtggtggt tgagggcaaa gaggttctct agctgatgac agaacagaag  
agattagcag  
3721 gctatcaaca cgtgggatgt attgagatgg ctccatggca cacttttgaa  
40 agataaaagt  
3781 gacttgctgg cgtggagcag agtctggccg aatgtcccta tctcagcggg  
ccattttgca  
3841 ctctctctct cccgagctta gtcacacctg gaccttggct gaagtttcca  
cagcatcgac

5	3901	gtgacccggg	tggggtggg	gtggggaagt	atgggtggtg	gttcgtggga
		tgttggttt				
	3961	gaccttttct	tccctcctcc	cctcgteccc	tectccccca	gaccgtgttc
10		tctgagatgc				
	4021	gccaagagtg	caaatgccac	gggatgtccg	gctcctgcac	ggtgcgcacg
		tgttggtatgc				
15	4081	ggctgcccac	gctgcgcgct	gtgggcgacg	tgctgcgcga	ccgcttcgac
		ggcgcctccc				
	4141	gcgtccttta	cggcaaccga	ggcagcaacc	gcgcctcgcg	ggcggagctg
20		ctgcgcctgg				
	4201	agcccgaaga	ccccgcgcac	aagcctccct	cccctcacga	cctcgtctac
		ttcgagaaat				
25	4261	cgcccaactt	ctgcacgtac	agtggccgcc	tgggcacagc	tggcacagct
		ggacgagctt				
	4321	gcaacagctc	gtctcccgcg	ctggacggct	gtgagctgct	gtgctgtggc
30		cgaggccacc				
	4381	gcacgcgcac	gcagcgcgtc	acggagcgct	gcaactgcac	cttcactgg
		tgctgccacg				
35	4441	tcagctgccg	caactgcacg	cacacgcgcg	ttctgcacga	gtgtctatga
		ggtgccgcgc				
	4501	ctccgggaac	gggaacgctc	tcttcagtt	ctcagacaca	ctcgttggtc
40		ctgatgtttg				
	4561	cccaccctac	cgcgtccagc	cacagtccca	gggttcatag	cgatccatct
		ctccacctc				
45	4621	ctacctgggg	actcctgaaa	ccacttgccct	gagtcggctc	gaaccctttt
		gccatcctga				
	4681	gggccctgac	ccagcctacc	tccctccctc	tttgagggag	actccttttg
50		cactgcccc				
	4741	caatttggcc	agagggtgag	agaaagattc	ttcttctggg	gtgggggtgg
		ggaggtcaac				
55	4801	tcttgaaggt	gttgcgggtc	ctgatgtatt	ttgcgctgtg	acctctttgg
		gtattatcac				
	4861	ctttccttgt	ctctcgggtc	cctataggct	ccttgagttc	tctaaccagc
60		acctctgggc				
	4921	ttcaaggcct	ttccccctcc	acctgtagct	gaagagtttc	cgagttgaaa
		gggcacggaa				
65	4981	agctaagtgg	gaaaggaggt	tgctggaccc	agcagcaaaa	ccctacattc
		tccttgctctc				
	5041	tgccctcggag	ccattgaaca	gctgtgaacc	atgcctccct	cagcctcctc
70		ccacccttc				
	5101	ctgtcctgcc	tcctcatcac	tgtgtaaata	atttgacccg	aaatgtggcc
		gcagagccac				
	5161	gcgttcgggt	atgtaaataa	aactatttat	tgtgctgggt	tccagcctgg
		gttgagaga				

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5221 ccaccctcac cccacctcac tgctcctctg ttctgctcgc cagtcctttt  
 gttatccgac  
 5281 cttttttctc ttttaccag cttctcatag gcgccttgcc ccaccggatc  
 agtatttctt  
 5 5341 tccactgtag ctattagtgg ctctcgcgcc ccaccaatgt agtatcttcc  
 tctgaggaat  
 5401 aaaatatcta tttttatcaa cgactctggc ccttgaatcc agaacacagc  
 atggcttcca  
 5461 acgtcctctt cccttccaat ggacttgctt ctcttctcat agccaaacaa  
 10 aagagataga  
 5521 gttgttgaag atctcttttc cagggcctga gcaaggaccc tgagatcctg  
 acccttgat  
 5581 gaccctaaat gagaccaact agggatc (SEQ ID NO:6)

Table 12: Human Wnt-2 Nucleotide Sequence

15 1 agcagagcgg acgggcgcgc gggaggcgcg cagagctttc gggctgcagg cgctcgctgc  
 61 cgctggggaa ttgggctgtg ggcgaggcgg tccgggctgg cctttatcgc tcgctgggcc  
 121 catcgtttga aactttatca gcgagtcgcc actcgctcga ggaccgagcg gggggcgggg  
 181 gcgcggcgag gcggcgcccg tgacgaggcg ctcccggagc tgagcgcttc tgctctgggc  
 241 acgcatggcg ccgcacacg gagtctgacc tgatgcagac gcaagggggg taatatgaac  
 20 301 gcccctctcg gtggaatctg gctctggctc cctctgctct tgacctggct cacccccag  
 361 gtcaactctt catggtggta catgagagct acaggtggct cctccagggt gatgtgcgat  
 421 aatgtgccag gcctggtgag cagccagcgg cagctgtgtc accgacatcc agatgtgatg  
 481 cgtgccatta gccaggcgt ggccgagtg acagcagaat gccagacca gttccgccag  
 541 caccgctgga attgcaacac cctggacagg gatcacagcc tttttggcag ggtcctactc  
 25 601 cgaagtagtc gggaaatctg ctttgtttat gccatctcct cagctggagt tgtatttgcc  
 661 atcaccaggg cctgtagcca aggagaagta aaatcctgtt cctgtgatcc aaagaagatg  
 721 ggaagcgcca aggacagcaa aggcattttt gattgggggt gctgcagtga taacattgac  
 781 tatgggatca aatttgcccg cgcattttgt gatgcaaagg aaaggaaagg aaaggatgcc  
 841 agagccctga tgaatcttca caacaacaga gctggcagga aggctgtaaa gcggttcttg  
 30 901 aaacaagagt gcaagtgcc aaggggtgag ggctcatgta ctctcaggac atgctggctg  
 961 gccatggcgg acttcaggaa aacgggcgat tatctctgga ggaagtacaa tggggccatc  
 1021 caggtggtca tgaaccagga tggcacagg ttcactgtgg ctaacgagag gttaagaag  
 1081 ccaacgaaaa atgacctcgt gtattttgag aattctccag actactgtat cagggaccga  
 1141 gaggcaggct ccctgggtac agcaggccgt gtgtgcaacc tgacttcccg gggcatggac  
 35 1201 agctgtgaag tcatgtgctg tgggagaggc tacgacacct cccatgtcac ccggatgacc  
 1261 aagtgtgggt gtaagttcca ctggtgctgc gccgtgcgt gtcaggactg cctggaagct  
 1321 ctggatgtgc acacatgcaa gggccccaag aacgctgact ggacaaccgc tacatgacct  
 1381 cagcaggcgt caccatccac cttcccttct acaaggactc cattggatct gcaagaacac  
 1441 tggacctttg ggttctttct ggggggatat ttcctaaggc atgtggcctt tatctcaacg  
 40 1501 gaagccccct cttcctccct gggggcccca ggatgggggg ccacacgctg cacctaaagc  
 1561 ctaccctatt ctatccatct cctggtgttc tgcagtcac tcccctcctg gcgagttctc

15	1	gaattcatgt	cttacgggtca	aggcagaggg	cccagcgcca	ctgcagccgc
		gccacctccc				
	61	agggccgggc	cagcccaggc	gtccgcgctc	tcggggtgga	ctccccccgc
		tgcgcgctca				
	121	agccggcgat	ggctcctctc	ggatacctct	tagtgctctg	cagcctgaag
20		caggctctgg				
	181	gcagctaccc	gatctgggtg	tccttggctg	tgggacccca	gtactcctct
		ctgagcactc				
	241	agcccattct	ctgtgccagc	atcccaggcc	tggtagccgaa	gcagctgcgc
		ttctgcagga				
25	301	actacgtgga	gatcatgccc	agcgtggctg	aggggtgtcaa	agcggggcatc
		caggagtgcc				
	361	agcaccagtt	ccgaggcccg	cgttggaaact	gcaccaccgt	cagcaacagc
		ctggccatct				
	421	ttggccctgt	tctggacaaa	gccacccggg	agtcagcctt	tgtccatgcc
30		atcgctccg				
	481	ctggagtagc	tttcgcagtg	acacgctcct	gtgcagaggg	atcagctgct
		atctgtgggt				
	541	gcagcagccg	cctccagggc	tcccaggcg	agggctggaa	gtggggcggc
		tgtagtgagg				
35	601	acattgaatt	tggaggaatg	gtctctcggg	agtttgccga	tgccagggag
		aaccggcccg				
	661	atgcccgctc	tgccatgaac	cgtcacaaca	atgaggctgg	gcgccaggcc
		atcgccagtc				
	721	acatgcacct	caagtgcaaa	tgccacgggc	tatctggcag	ctgtgaagtg
40		aagacctgct				
	781	ggtaggtcgca	gccggacttc	cgcaccatcg	gggatttcct	caaggacaag
		tatgacagtg				
	841	cctcgagat	ggtaggtagag	aaacaccgag	agtctcgtgg	ctgggtggag

	accctgaggc				
	901	cacgttacac	gtacttcaag	gtgccgacag	aacgcgacct
		gaggcctcac			ggtctactac
	961	ccaacttctg	cgaacctaac	cccgaaccg	gctccttcgg
5		cgcacctgca			gacgcgtgac
	1021	atgtgagctc	gcatggcata	gatgggtgcg	acctgttgtg
		gggcataacg			ctgcgggcgc
	1081	cgcgcactga	gcgacggagg	gagaaatgcc	actgtgtttt
		tgctacgtca			ccattggtgc
10		1141	gctgccagga	gtgcacacgt	gtctatgacg
			agctcctaac		tgcacacctg
		1201	acgggagcag	ggttcattcc	gagtaggag
			ttctacttg		caagtaggag
		1261	gaggggtctc	ttacttgggg	gttcctacct
15			acctgtgagg		gggggcgggg
		1321	gtctcatacc	taaggaccgc	gttcctacct
			ggatctgggt		gggggcgggg
		1381	tccttttttag	gggagaagct	gttcctacct
			gtggggctcc		gggggcgggg
20		1441	acttggggat	ggaattccaa	gttcctacct
			ggactcctct		gggggcgggg
		1501	cttgaccgca	cagggtcaa	gttcctacct
			ggtgggggttc		gggggcgggg
		1561	gtgcggatgg	gtgggagggg	gttcctacct
25			tgctctatct		gggggcgggg
		1621	agatacatga	gaggggtgctt	gttcctacct
			ccgtggggggc		gggggcgggg
		1681	ggggcttcac	cccgactggg	gttcctacct
			gcaaggcttc		gggggcgggg
30		1741	actgaagact	catgggatgg	gttcctacct
			gcctgggctc		gggggcgggg
		1801	tgagcaggcc	atccagctcc	gttcctacct
			gttcaacctg		gggggcgggg
		1861	caagcctcat	ctgcgcagag	gttcctacct
35			aagaactcag		gggggcgggg
		1921	gggtgatacc	aagacctaac	gttcctacct
			ctctgcaccc		gggggcgggg
		1981	cttcttcaag	ggcttttcta	gttcctacct
			tttgagtc		gggggcgggg
40		2041	cccagagttc	aagtgaacac	gttcctacct
			agagagggtt		gggggcgggg
		2101	ctctaggaat	ctctatgggg	gttcctacct
			ctcgtatgat		gggggcgggg
		2161	aqcctgcac	cgtctgcaca	gttcctacct
					gggggcgggg

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ctcatccggt
2221 ccgtgatgtc catgccccaa atgcctcaga gatgttgctt cactttgagt
tgtatgaact
2281 tcggagacat ggggacacag tcaagccgca gagccagggg tgtttcagga
5 cccatctgat
2341 tccccagagc ctgctgttga ggcaatgggc accagatccg ttggccacca
ccctgtcccg
2401 agctttctta gtgtctgtct ggcttgaag tgagggtgcta catacagccc
atctgccaca
10 2461 agagcttctt gattgggtacc actgtgaacc gtccctcccc ctccagacag
gggaggggat
2521 gtggccatac aggagtgtgc cgggagagcg cggaaagagg aagagaggct
gcacacgcgt
2581 ggtgactgac tgtcttctgc ctggaacttt gcgttcgcgc ttgtaacttt
15 attttcaatg
2641 ctgctatata caccaccac tggatttaga caaaagtgat tttctttttt
tttttttttt
2701 ttctttctat gaaagaaatt attttagttt atagtatgtt tgtttcaaat
aatggggaaa
20 2761 gtaaaaagag agaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaa
(SEQ ID NO:8)

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Table 14: Human Wnt-3a nucleotide sequence

```

tgtaagtgcc acgggctgtc gggcagctgc gaggtgaaga catgctggtg
gtcgcaacce gacttccgcg ccatcgggtg attcctcaag gacaagtacg
25 acagcgcttc ggagatggtg gtggagaagc accgggagtc ccgcggctgg
gtggagaccc tgcggccgcg ctacacctac ttcaagggtc ccacggagcg
cgacctggtc tactacgagg cctcgcccaa cttctgcgag cccaacctg
agacgggctc cttcggcacg cgcgaccgca cctgcaacgt cagctcgcac
ggcatcgacg gctgcgacct gctgtgctgc ggccgcggcc acaacgcgcg
30 agcggagcgg cgccgggaga agtgccgctg cgtgtttcac tgggtgctgt
(SEQ ID NO:9)

```

Human nucleic acid sequences which encode Wnt-4, Wnt-7a, and Wnt-7b are shown Tables 15, 16, and 17, respectively.

Human and mouse Wnt polypeptides function similarly in transformation assays. Accordingly, human or mouse Wnt polypeptides or nucleic acids are administered to mammals to therapeutically stimulate

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tubulogenesis. The amino acid and nucleotide sequences of Wnt polypeptides are known in the art, e.g., human Wnt-1 (GENBANK® X03072), human Wnt-2 (GENBANK® X07876), human Wnt-4 (GENBANK® AAB30677), human Wnt-7a (GENBANK® 000755), mouse Wnt-1 (GENBANK® P04426), mouse Wnt-2 (GENBANK® P21552), mouse Wnt-3a (GENBANK® P27467), mouse Wnt-4 (GENBANK® P22724 and M89787), mouse Wnt-7a (GENBANK® M89802), and mouse Wnt-7a (GENBANK® M89801).

Kidney tubulogenesis is a multi-step process with a hierarchy of signaling systems. A permissive signal from the ureter to the mesenchyme triggers survival and tubulogenesis in the mesenchyme, signals from the mesenchyme to the ureter are required for proliferation and branching morphogenesis of the ureter. Other signaling systems within the ureter are required for local adhesion and proliferation, changes which may mediate branching morphogenesis, and within the mesenchyme, for tubulogenesis as evidenced by the role of Wnt-4.

The data described herein indicate that Wnt-4 is sufficient to trigger tubulogenesis in isolated metanephric mesenchyme, whereas Wnt-11 which is expressed in the tip of the growing ureter is not. Wnt-4 signaling depends on cell contact and sulphated glycosaminoglycans. Wnt-4 is required for triggering tubulogenesis but not for later developmental events. The Wnt-4 signal can be replaced by other members of the Wnt gene family including Wnt-1, Wnt-3a, Wnt-7a and Wnt-7b. Further, dorsal spinal cord, which has been thought to mimic ureteric signaling in tubule induction, induces Wnt-4 mutant as well as wild-type mesenchyme suggesting that spinal cord derived signal(s) likely act by mimicking the normal mesenchymal action of Wnt-4. These results indicate that Wnt-4 is a key auto-regulator of the mesenchymal to epithelial transformation that leads to

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tubulogenesis and nephrogenesis.

Therapeutic administration of a Wnt polypeptide or agonist

- Wnt polypeptides or agonists are useful to treat
- 5 kidney disorders such as chronic renal insufficiency, end-stage chronic renal failure, glomerulonephritis, glomerulosclerosis, interstitial nephritis, pyelonephritis, kidney failure due to viral disease, kidney failure after transplantation.
- 10 Wnt polypeptides are at least about 10 amino acids, usually about 20 contiguous amino acids, preferably at least 40 contiguous amino acids, more preferably at least 50 contiguous amino acids, and most preferably at least about 60 to 80 contiguous amino acids
- 15 in length and have the biological activity of triggering tubulogenesis. For example, a Wnt polypeptide is at least 50% of the length of the corresponding naturally-occurring Wnt polypeptide and has the amino acid sequences of the amino-terminal half of the naturally-
- 20 occurring polypeptide. Such peptides are generated by methods known to those skilled in the art, including proteolytic cleavage of the protein, *de novo* synthesis of the fragment, or genetic engineering, e.g., cloning and expression of a fragment of Wnt-encoding cDNA.
- 25 Therapeutic compositions are administered in a pharmaceutically acceptable carrier (e.g., physiological saline). Carriers are selected on the basis of mode and route of administration and standard pharmaceutical practice. A therapeutically effective amount of a
- 30 composition (e.g., Wnt polypeptide or agonist) is an amount which is capable of producing a medically desirable result, e.g., tubulogenesis, in a treated animal. As is well known in the medical arts, dosage for any one animal depends on many factors, including the
- 35 animal's size, body surface area, age, the particular

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compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently (e.g., other Wnt polypeptides) is 0.1 to 100 mg/kg body weight. Administration is generally be parenterally, e.g., intravenously, subcutaneously, intramuscularly, or intraperitoneally. The compositions of the invention can be administered locally i.e., at the site of organ damage or systemically. For example, the route of delivery is by intravenous infusion, localized injection or implants. The polypeptides or agonists may be formulated so as to have a continual presence in the tissue during the course of treatment, e.g., by being covalently attached to a polymer such as polyethylene glycol (PEG). Such continuous release formulations are administered at weekly intervals or at multiples of weekly intervals. Examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the polypeptide or agonist, which matrices are in the form of shaped films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (e.g., poly(2-hydroxyethyl-methacrylate) as described by Langer et al., 1981, J. Biomed. Mater. Res., 15: 167-277 and Langer, 1982, Chem. Tech., 12: 98-105 or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., 1983, Biopolymers, 22: 547-556), non-degradable ethylene-vinyl acetate (Langer et al., *supra*), degradable lactic acid-glycolic acid copolymers, polylactate polyglycolate (PLGA), and poly-D-(-)-3-hydroxybutyric acid (EP 133,988). While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid provide release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

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Sustained-release Wnt compositions also include liposomally entrapped Wnt polypeptides or agonists. Liposomes containing Wnt compositions are prepared by methods known in the art, e.g., Epstein et al., 1985, 5 Proc. Natl. Acad. Sci. USA, 82: 3688-3692; Hwang et al., 1980, Proc. Natl. Acad. Sci. USA, 77: 4030-4034; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. The compositions may also be administered directly to a tissue site, e.g., by biolistic delivery to an internal 10 or external target site or by catheter into a body lumen. Therapeutic compositions are administered by retrograde perfusion of kidney via the ureter or other urine collecting lumens, e.g., using a catheter or perfusion apparatus, such as that described in U.S. Pat. No. 15 5,871,464.

Analog, homologs, or mimetics of the above peptides may also be used to induce and promote kidney tubule formation in a post-natal mammal. Analog can differ from the naturally-occurring Wnt polypeptides by 20 amino acid sequence, or by modifications which do not affect the sequence, or both. Modifications (which do not normally alter primary sequence) include *in vivo* or *in vitro* chemical derivitization of polypeptides, e.g., acetylation or carboxylation. Also included are 25 modifications of glycosylation, e.g., those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps, e.g., by exposing the polypeptide to enzymes which affect glycosylation, e.g., mammalian 30 glycosylating or deglycosylating enzymes. To improve the solubility and therapeutic half-life of Wnt polypeptides, Wnt-Ig fusion proteins are produced. Methods of making Ig fusion proteins is well known in the art (e.g., as described in Current Protocols of Immunology, 1994, 35 Coligan et al., eds., John Wiley & Sons, Inc., p.

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10.19.1-10.19.11).

To render the therapeutic peptides less susceptible to cleavage by peptidases, the peptide bonds of a peptide may be replaced with an alternative type of covalent bond (a "peptide mimetic"). Where proteolytic degradation of the peptides following injection into the subject is a problem, replacement of a particularly sensitive peptide bond with a noncleavable peptide mimetic renders the resulting peptide more stable, and thus more useful as a therapeutic. Such mimetics, and methods of incorporating them into polypeptides, are well known in the art. Similarly, the replacement of an L-amino acid residue with a D-amino acid is a standard way of rendering the polypeptide less sensitive to proteolysis. Also useful are amino-terminal blocking groups such as t-butyloxycarbonyl, acetyl, theryl, succinyl, methoxysuccinyl, suberyl, adipyl, azelayl, dansyl, benzyloxycarbonyl, fluorenylmethoxycarbonyl, methoxyazelayl, methoxyadipyl, methoxysuberyl, and 2,4,-dinitrophenyl. Peptides may be administered to a subject intravenously in a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are biologically compatible vehicles which are suitable for administration to an animal: e.g., physiological saline.

Wnt polypeptides are generally administered *in vivo* to allow regeneration of kidney tissue in the context of the autologous organ. However, kidney tissue or dissociated cells (derived from kidney tissue or embryonic tissue) may be treated outside the body (i.e., *ex vivo*) and then transplanted back into the body from which it was derived or into a different mammal. In the case of *ex vivo* therapy, a damaged or diseased kidney is removed from an individual, treated with a Wnt polypeptide (or DNA encoding a Wnt polypeptide) and then transplanted into the same individual or a different

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individual.

Therapeutic administration of DNA encoding a Wnt polypeptide or agonist

Gene therapy for regeneration of kidney tissue is carried out by directly administering the claimed DNA to a mammal or by transfecting kidney cells, e.g., renal mesenchymal cells or endothelial cells, with Wnt-encoding DNA *in vivo* or *ex vivo*. Gene transfer into kidney tissue is carried out using known methods, e.g., bathing the tissue or cells in a solution containing Wnt-encoding DNA. Alternatively, kidney tissue is perfused *in vivo* or explanted kidney tissue is perfused *ex vivo*, using a perfusion apparatus, such as that described in U.S. Pat. No. 5,871,464. After the cells are contacted with DNA, the cells or organ is transplanted into a recipient (or returned to the host from which it was removed). If the cells in suspension, the cells are infused into the mammal to be treated.

To express a Wnt polypeptide in a kidney cell, a Wnt-encoding DNA is introduced into a target cell, e.g., a mesenchymal or epithelial kidney cell, of the mammal by standard vectors and/or gene delivery systems. For example, expression of exogenous Wnt DNA in an epithelial cell induces production and secretion of a Wnt polypeptide, which in turn, leads to tubulogenesis and kidney regeneration. Suitable gene delivery systems may include liposomes, receptor-mediated delivery systems, naked DNA, and viral vectors such as herpes viruses, retroviruses, adenovirus, and adeno-associated virus, among others. A therapeutically effective amount is an amount of the nucleic acid of the invention which is capable of producing a medically desirable result in a treated animal, e.g., tubulogenesis.

DNA or transfected cells may be administered in a pharmaceutically acceptable carrier. Pharmaceutically

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acceptable carriers are biologically compatible vehicles which are suitable for administration to a mammal, e.g., physiological saline. As is well known in the medical arts, dosages for any one patient depends upon many  
5 factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosages will vary, but a preferred dosage for intravenous  
10 administration of DNA is from approximately  $10^6$  to  $10^{22}$  copies of the DNA molecule. The compositions of the invention may be administered locally or systemically. As with other therapeutic compositions such as peptides, administration of a nucleic acid composition is generally  
15 be parenterally, e.g., intravenously. DNA is also administered by retrograde perfusion of kidney tissue using, e.g., a catheter. DNA may also be administered directly to the target site, e.g., by biolistic delivery to a kidney tissue or by an implantable device.

20 Methods of delivering nucleic acids to kidney tissue are known in the art, e.g, those described by Sukhatme et al. in U.S. Pat. No. 5,869,230. Nucleic acids are expressed under the control of tissue-specific, e.g., kidney-specific, promoters such as the Pax-2  
25 promoter, the cRET promoter, and the Hox b7 promoter. Promoter constructs for inducible and constitutive expression of heterologous sequences are well known in the art and commercially-available. For example, nucleic acids are expressed under the control of the  
30 cytomegalovirus (CMV)  $\beta$ -actin promoter for general constitutive expression.

Method of screening for compounds which increase Wnt expression

A screening assay to identify compounds which are  
35 capable of inducing or increasing Wnt polypeptide

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expression in kidney tissue of a post-natal mammal (i.e., non-embryonic cells) is carried out as follows. For example, a sample of kidney cells, e.g., cultured mesenchymal or epithelial cells, is incubated in the presence of a candidate compound. A sample of control cells is incubated in the absence of the compound. Each sample of cells is evaluated for the expression of a Wnt polypeptide, e.g., Wnt-4. To test for presence of the Wnt gene product, each sample of cells can be incubated with a Wnt-specific antibody and the cells evaluated for binding of the antibody by methods well known in the art, e.g., immunofluorescent staining. The amount of antibody binding correlates with the level of expression of the Wnt polypeptide. Wnt expression is also measured at the level of gene transcription. For example, Wnt transcripts can be measured by Northern blotting techniques using Wnt-specific DNA probes or by PCR using Wnt-specific DNA primers. A increase in the amount of Wnt gene expression in cells contacted with a candidate compound compared to the amount in untreated cells indicates that the candidate compound is capable of inducing or increasing the expression of a Wnt polypeptide in kidney cells (and inducing tubulogenesis). The compound is tested in tissue or organ culture systems as described below to determine whether the compound triggers tubulogenesis.

#### Mouse model of renal development

Mouse renal development is characterized by the continuous interaction of epithelial and mesenchymal compartments both of which are derived from the intermediate mesenchyme. These compartments are the nephric duct and its derivative, the ureter, and the nephrogenic mesenchyme which lies adjacent to these ducts. As a consequence of these interactions, three embryonic kidneys are laid down from anterior to

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posterior in time and space. While the initial organ, the pronephros is only a very transient structure established at 8-8.5 days post coitum (d.p.c.), the mesonephros extends by posterior elongation of the nephric duct and subsequent tubule induction in the adjacent mesonephrogenic mesenchyme between 9 and 11 d p.c. Although forming elaborate tubules, the mesonephros of the male never becomes a functional organ but contributes to the ductal network of the rete testis.

Metanephric development is initiated when a bud emerges from the nephric duct at the level of the hind limbs around 10.5 d.p.c. The ureteric duct subsequently invades the metanephric blastema which lies at the posterior end of the intermediate mesoderm.

In a process repeated many times, mesenchymal cells condense around the tip of the ureter, i.e., bud, aggregate, epithelialize and undergo morphogenetic movements. Cellular differentiation occurs to form a major part of the nephron, the functional unit of the vertebrate kidney. The ureter continues to grow and to branch forming the collecting duct system of the mature organ. 7-10 days post partum, nephron formation ceases as the mesenchymal stem cells in the periphery of the kidney are exhausted.

The role of Wnt-11, Wnt-4 and other Wnt family members in tubule induction was studied. Wnt-4, but not Wnt-11 was found to be able to induce tubule formation, suggesting that spinal cord mediated tubulogenesis reflects the normal mesenchymal function of Wnt-4 rather than that of a ureteric bud derived signal.

The following reagents and procedures were used to evaluate Wnt signalling in the developing kidney.

#### Mice

Wnt-4 heterozygotes were derived and genotyped using known methods, e.g., that described by Stark et

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al., 1994, Nature 372:679-683. Embryos for kidney dissections were derived from matings of Swiss Webster (SW) wild-type animals or Wnt-4 heterozygotes. For timed pregnancies, plugs were checked in the morning after mating, noon was taken as 0.5 d.p.c.

#### Cell lines

Cell lines which stably express various Wnt genes or LacZ were prepared using standard methods, e.g., that described by Pear et al., 1993, Proc. Natl. Acad. Sci. USA 90: 8392-8396. For Wnt polypeptide expression, full-length cDNAs encoding Wnt-1 (van Ooyen and Nusse, 1984, Cell 39: 233-240), Wnt-3a (Roelink and Nusse, 1991, Genes Dev. 5: 381-388), Wnt-4, Wnt-5a, Wnt-7a, Wnt-7b (Gavin et al., 1990, Genes Dev. 4: 2319-2332), Wnt-11 (Kispert et al., 1996, Development 122:3627-3637) and lacZ were cloned into an expression vector, e.g., the retroviral expression vector pLNCX which confers expression of foreign genes under the control of the CMV promotor. Bosc23 packaging cells were transfected with recombinant DNA constructs. Viral supernatants were collected 48-72 h later and used to infect standard NIH3T3 cells. After 10 d of selection in G418, pools of cells were used for recombination experiments. 50,000 cells were plated in 50  $\mu$ l of medium on polycarbonate filter and grown for 18-24 h at 37°C in 5% CO<sub>2</sub>.

#### Organ culture techniques

Metanephric kidneys from SW or Wnt-4 intercrosses were dissected in phosphate buffered saline (PBS). To generate a preparation of dissociated kidney cells from embryonic or mature tissue, the tissue is dissected and enzymatically digested. For example, metanephric mesenchyme was dissected manually from the ureter (bud stage [10.75 d.p.c.] to early T stage [11.5 d.p.c.]), following a 2 min. incubation in 3% pancreatin/trypsin (GibcoBRL) in Tyrode's solution. In recombination

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experiments with wild-type mesenchymes, samples were pooled before being distributed to individual experiments. In experiments with Wnt-4 mutant embryos, metanephric mesenchyme from each kidney of the embryo was kept separate. The remainder of an embryo was used for genotyping by Southern analysis. In recombination experiments with dorsal spinal cord, metanephric mesenchyme from two kidneys was surrounded by two dissected pieces of dorsal spinal cord from the same embryo on a 1  $\mu$ m polycarbonate filter (Costar). For direct recombination experiments with Wnt-expressing cells, two mesenchymes were placed on top of modified NIH3T3 cells. For transfilter experiments, 50,000 cells in 50  $\mu$ l medium were seeded on a 1  $\mu$ m filter 18-24 h prior to the recombination. Cells were then covered with a 1  $\mu$ m filter and two mesenchymes placed on this filter. Filters (4-6 mm in size) were supported by stainless steel grids on the surface of the culture medium (Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM glutamine, 1  $\times$  penicillin/streptomycin). Medium was changed every 2 d. For studies of glycosaminoglycan dependence of tubule induction, the medium was supplemented with 30 mM NaClO<sub>3</sub> after 0 h, 24 h and 48 h, respectively. In experiments concerning pore size dependence of induction, the pore size of the upper filter in the transfilter set-up was varied from 0.05  $\mu$ m, 0.1  $\mu$ m, 0.4  $\mu$ m, 0.8  $\mu$ m to 1  $\mu$ m. For marker experiments, at least 6 specimens were processed.

For *in situ* hybridization analysis, filters were submerged in cold methanol for 10 seconds and then fixed in 4% paraformaldehyde in PBS overnight prior to stepwise transfer into methanol and storage at -20°C. For histological analysis, filters were fixed in Bouin's solution and stored in 70% ethanol at 4°C.

In situ hybridization analysis

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*In situ* hybridization analysis on whole mount cultures were performed using standard methods. Full-length cDNAs for WT-1 (Pritchard-Jones et al., 1990, Nature 346:194-197), Pax-2 (Dressler et al., 1990, Development 109:787-795), Pax-8 (Plachov et al., 1990, Development 110:643-651), Wnt-4 (Gavin et al., 1990, Genes Dev. 4:2319-2332) and E-cadherin (Ringwald et al., 1987, EMBO J. 6:3647-3653) were labeled with Digoxigenin for whole mount detection.

#### 10 Histological analysis and documentation

Samples were dehydrated, embedded in wax and sectioned at 5  $\mu$ m. Sections were dewaxed, rehydrated and stained with haematoxylin and eosin. Brightfield images of cultures and marker stainings were taken with a binocular on Kodak 64T slide film. Histological sections were photographed on the same film on a Leitz Axiophot. Slides were scanned and documented in Adobe Photoshop 4.0.

#### 20 Spinal cord mimics a mesenchymal signal for tubule induction

The identification of Wnt-4 as a mesenchymal signal essential for tubule formation provides a strategy for evaluating the role of spinal cord explants as heterologous inducers of kidney tubulogenesis. If the spinal cord mimics a ureteric signal upstream of Wnt-4, this signal would not rescue the mesenchymal requirement for Wnt-4 in tubulogenesis. To test this possibility, isolated metanephric mesenchyme from individual embryos derived from intercrosses between mice heterozygous for a likely null allele of Wnt-4 were cultured on a polycarbonate filter in direct contact with dorsal spinal cord from the same embryo. In the absence of spinal cord, all mesenchyme cultures rapidly degenerated as expected. Surprisingly, when cultured in the presence of spinal cord, mesenchyme from Wnt-4 mutant embryos

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developed as well as that of wild-type or heterozygous siblings (Table 1).

Table 1: Induction of tubulogenesis in Wnt-4/Wnt-4 mutant metanephric mesenchyme by dorsal spinal cord

5	<u>Exp #</u>	<u># Recombinants</u>	<u>#induced/#total</u>		
			+/+	Wnt-4/+	Wnt-4/Wnt-4
	1	8	2/2	5/5	1/1
	2	7	1/1	3/3	3/3
	3	7	3/3	3/3	1/1
10	4	5	1/1	3/3	1/1
	5	9	3/3	4/4	2/2
	6	11	7/7	4/4	-
	7	11	3/3	4/4	4/4
	Total	58	20/20	26/26	12/12

15 Isolated metanephric mesenchyme was recombined with dorsal spinal cord from the same embryo on a nucleopore filter. Induction was monitored by bright field microscopy. Embryos of a total of seven litters were analyzed.

20 Induction of tubulogenesis in wild-type and Wnt-4 mutant metanephric mesenchyme by dorsal spinal cord was analyzed as follows. Isolated metanephric mesenchyme and dorsal spinal cord from the same 11.5 d embryo were recombined on a nucleopore filter. After 48 h and 96 h, 25 cultures were monitored as whole mounts using bright field microscopy; after 144 h, they were analyzed as histological sections. Induction of tubulogenesis in wild-type and Wnt-4/Wnt-4 mutant metanephric mesenchyme were indistinguishable.

30 After 48 h, induction was visible as bright round zones of condensing mesenchyme. After 96 h, the zones of

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condensing mesenchyme had undergone epithelialization to form complex tubules. At 144 h, epithelial tubular structures and glomeruli indicated that full differentiation of induced tubules occurred in all 5 recombinants.

The induction of tubulogenesis in Wnt-4 mutant mesenchyme indicates that spinal cord signaling acts by either mimicking the action of Wnt-4 itself, or a factor downstream of Wnt-4. Further, although Wnt-4 is 10 expressed in the spinal cord, the observation that spinal cord from Wnt-4 mutants is capable of induction indicates that Wnt-4 expression in the spinal cord is not essential for this process, suggesting the involvement of other Wnts expressed in this tissue.

15 Wnt polypeptides which are sufficient to trigger tubulogenesis

In order to investigate whether Wnt-4 is sufficient for tubulogenesis, and if this property is shared by other Wnts normally expressed in the spinal 20 cord, NIH3T3 cell lines which stably express various Wnt genes were established. Direct recombinations were performed between Wnt-expressing cells and isolated wild-type metanephric mesenchyme.

Isolated metanephric mesenchyme from 2-3 11.5 d 25 kidneys was placed on top of NIH3T3 cells expressing various Wnt genes. As a control, mesenchymes were placed on NIH3T3 cells expressing LacZ and were placed onto a filter without an underlying cell layer. Induction was scored after 6 d using the morphological appearance of 30 the culture (as documented by brightfield microscopy), and histological analysis of selected samples. For each cell type 2-3 independent experiments were performed.

Induction of tubulogenesis in isolated metanephric mesenchyme by NIH3T3 cells expressing various Wnt genes 35 was evaluated as follows. Brightfield microscopy (24 h,

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88 h) and histological analysis (144 h) of direct recombinations between NIH3T3 cells expressing Wnt genes and isolated metanephric mesenchyme. After 24 h, bright zones indicating induction were visible in recombinants  
 5 between wild-type mesenchyme and Wnt-1, Wnt-3a, Wnt-4, Wnt-7a and Wnt-7b expressing cells. These condensing mesenchymal cells had epithelialized and formed tubular structures after 88 h. After 144 h highly elaborate tubular structures were apparent. In contrast, cells  
 10 expressing Wnt-5a, Wnt-11, or as a control lacZ, respectively, did not support survival and differentiation of metanephric mesenchyme.

Co-cultures with Wnt-1, Wnt-3a, Wnt-4, Wnt-7a and Wnt-7b expressing cells developed on schedule with those  
 15 induced by spinal cord and formed complex epithelial tubules with differentiated glomeruli at 144 h (Table 2). In contrast, cells expressing Wnt-5a, Wnt-11 or a lacZ control did not support survival and differentiation of metanephric mesenchyme (Table 2).

20 Table 2: Induction of tubulogenesis in isolated metanephric mesenchyme by NIH3T3 cells expressing various Wnt genes

	Cell line	#induced/#total
25	Wnt-1	16/16
	Wnt-3a	14/14
	Wnt-4	14/14
	Wnt-5a	0/12
	Wnt-7a	12/12
	Wnt-7b	11/12
30	Wnt-11	0/12
	LacZ	1/14
	mesenchyme	1/12
	Wnt mRNA expression was comparable amongst the	

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various lines. These data indicate that a subset of Wnt genes, which includes Wnt-4 and not Wnt-11, induces tubule formation. As all of these are expressed in the spinal cord at the time of assay, it is likely that these  
5 signals account for the robust inducing activity of the spinal cord. However, of these Wnt-4 is the only member which is actually expressed in and which is also required for mesenchymal aggregation.

10 Wnt-4 triggers the complete program of tubular differentiation

In order to investigate whether Wnt-4 is sufficient to induce fully developed tubules in isolated metanephric mesenchyme, the induction properties of NIH3T3 cells expressing Wnt-4 were analyzed more  
15 carefully by assessing the differentiation state of the mesenchyme by histological and molecular criteria.

Histological analysis of tubule induction in isolated metanephric mesenchyme by NIH3T3 cells expressing Wnt-4 was evaluated as follows. NIH3T3 cells  
20 expressing Wnt-4 were recombined with isolated metanephric mesenchyme directly and in a transfilter set-up. Cultures were analyzed by sectioning and histological staining after 24 h, 48 h, 96 h and 192 h of culture. Tubule induction in transfilter assays appeared  
25 slightly delayed compared to direct recombinations.

After 48 h, zones of condensed and aggregated mesenchyme were detected, and after 96 h, epithelial tubules were apparent. After 8 d in culture, fully differentiated tubular structures including glomeruli were detected.

30 Tubule induction by spinal cord was demonstrated in the art-recognized system in which cells are cultured with polycarbonate filters of a certain pore size (e.g., the method described by Grobstein, 1956, Science 118:52-55). Wnt-4-expressing cells were seeded on one filter;  
35 these cells were separated from isolated mesenchyme by

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another filter of 1  $\mu$ m pore size. Induction took place transfilter, though with a delay when compared with direct recombinants.

Transfilter cultures appeared less compact and flatter. Zones of condensed mesenchyme formed after 24 h, and aggregating mesenchyme and simple epithelial bodies appeared after 48 h. Epithelial tubules were seen after 96 h, and glomeruli were detected by 8 days.

To verify that these morphological features reflected an underlying differentiation of the mesenchyme in response to Wnt-4, the temporal and spatial expression of a number of molecular markers was examined. Marker analysis of tubule induction in isolated metanephric mesenchyme by NIH3T3 cells expressing Wnt-4 were analyzed as follows. NIH3T3 cells expressing Wnt-4 were recombined with isolated metanephric mesenchyme in a transfilter set-up and scored for marker expression by *in situ* analysis after 24 h, 48 h, 96 h and 192 h of culture, respectively. Expression of WT-1, Pax-2, Pax-8, Wnt-4 and E-cadherin, respectively, were in accordance with expression data from *in vivo* and *in vitro* studies of tubular differentiation.

WT-1 was broadly expressed after 1 d refining to small intensely labeled foci by 8 days of culture. This expression profile parallels the expression of this gene during metanephric development which is first expressed in condensing mesenchyme, then in simple epithelial bodies before it is restricted to podocytes in the glomeruli. In the recombinants, WT-1 expression was detected in glomeruli after 8 d in agreement with the histological analysis. Like WT-1, Pax-2 is also broadly expressed after 1 d, but becomes restricted to epithelial bodies and is lost after 4 d reflecting initial expression in condensing metanephric mesenchyme, continuing expression in simple epithelial bodies and

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subsequent down-regulation as glomeruli start to differentiate. Wnt-4 is expressed in aggregating mesenchyme, in the epithelial bodies which they generate and is subsequently down-regulated as these mature into S-shaped bodies. Pax-8, a paired-box transcription factor, has a similar early expression to Wnt-4 which has been shown to depend on Wnt-4 activity. In cultures, Wnt-4 was transiently expressed between 24 h and 96 h, peaking at 48 h. Pax-8 expression extended longer in S-shaped bodies. E-cadherin, which is expressed in the proximal tubules *in vivo*, was present after 24 h and was maintained, consistent with the differentiation of epithelial tubules along the proximal distal axis.

These data indicate that tubulogenesis in isolated metanephric mesenchyme induced by Wnt-4 follows a similar progression to that observed in the metanephric kidney *in vivo*. At the stage at which the metanephric mesenchyme (T-stage of the ureter) was isolated, initial ureteric signaling had occurred, as evidenced by the condensation of mesenchyme around the tip of the ureteric bud. However, this alone is insufficient to support mesenchymal survival and tubulogenesis. In contrast, Wnt-4 expressing cells were sufficient to support these processes. In order to exclude that Wnt-4 only maintains Wnt-4 expression in the isolated mesenchyme, mesenchyme derived from 10.75 d.p.c. embryos was also analyzed. At this stage, the ureter bud had just emerged and the metanephric mesenchyme can first be identified. Wnt-4 expressing cells triggered the complete differentiation program as judged by brightfield observation (12 out of 12 cases) and by molecular criteria (Pax-8 induction in 8 out of 8 cases after 4 d of culture).

Wnt-4 signaling requires cell contact

Tubule induction in isolated metanephric mesenchyme was analyzed with respect to filter pore size.

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Experiments using the spinal cord as a heterologous inducer suggest a requirement for cell-cell contact as pore sizes below 0.1  $\mu\text{m}$ , which prevent the extension of cytoplasmic processes, block induction.

5 Pore size dependence of tubule induction by Wnt-4 expressing cells was tested as follows. NIH3T3 cells expressing Wnt-4 were recombined with isolated metanephric mesenchyme in a transfilter set-up with various pore sizes of the nucleopore filter. Induction  
10 was scored after 4 d by Pax-8 expression in whole mount *in situ* analysis. Pore sizes of 0.1  $\mu\text{m}$  and bigger supported full induction of metanephric mesenchyme, whereas 0.05  $\mu\text{m}$  pore size reduced or abolished induction (Table 3).

15 Table 3: Induction of tubulogenesis in isolated metanephric mesenchyme by NIH3T3 cells expressing Wnt-4 in transfilter assays with increasing pore size

	<u>Pore size</u>	<u># induced/# total</u>
	0.05 $\mu\text{m}$	3*/13
20	0.1 $\mu\text{m}$	14/16
	0.4 $\mu\text{m}$	14/14
	0.8 $\mu\text{m}$	6/6
	1 $\mu\text{m}$	3/3

\* In each of the specimen scored as induced, only 1-4  
25 spots of Pax-8 expression were seen in contrast to 15-30 with all the other pore sizes.

Supernatants from Wnt-4 expressing cells alone did not induce tubulogenesis, suggesting that cell contact is required. Wnt-4 may act as an insoluble cell bound  
30 factor or it may associate with the extracellular matrix (ECM). It is unlikely that Wnt-4 mediated induction occurs through a secondary, soluble factor.

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Wnt-4 signaling requires sulphated glycosaminoglycans

Experiments were carried out to determine whether Wnt signaling for tubule induction depends on sulfated glycosaminoglycans (GAG)s which might act as cofactors for binding the Wnt protein on the responsive cell. Accordingly, studies were undertaken to see evaluate whether the presence of 30 mM NaClO<sub>3</sub> (a competitive inhibitor of sulfation of GAGs) affects tubule induction. NIH3T3 cells expressing Wnt-4 were recombined with isolated metanephric mesenchyme in a transfilter set-up with addition of 30 mM NaClO<sub>3</sub> in the medium. NaClO<sub>3</sub> was added to cultures at the start of transfilter culture, or 24 and 48 h later. As a control, chlorate was omitted completely. Induction was scored after 4 d by Pax-8 expression using whole mount *in situ* hybridization analysis. Addition of 30 mM NaClO<sub>3</sub> after 24 h or 48 h of culture did not affect tubule induction compared to untreated controls, whereas administration of 30 mM NaClO<sub>3</sub> at the beginning of the culture abrogated tubule induction completely (Table 4).

Table 4: Induction of tubulogenesis in isolated metanephric mesenchyme by NIH3T3 cells expressing Wnt-4 in presence of 30 mM NaClO<sub>3</sub>

30 mM NaClO <sub>3</sub> added	
after h in culture	#induced/#total
0 h	0/19
24 h	12/19
48 h	14/17
-	12/15

When chlorate was added at 0 h, mesenchyme degenerated and Pax-8 expression was consequently negative. However, addition of chlorate after 24 h did

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not influence Pax-8 expression. Hence, GAGs are not involved in tubule maturation and differentiation. Tubule induction does, however, depend on sulfated GAGs in the first 24 h, the period essential for complete  
5 induction by the spinal cord.

The chlorate inhibition experiments define a critical period of 24 h for induction. Further differentiation, i.e. aggregation and epithelialization of mesenchymal cells is only initiated when a certain  
10 number of cells (a small community) has received the Wnt-4 signal. At this time, mesenchymal development is independent of ureteric signaling.

Chlorate acts as a competitive inhibitor of sulphotransferases and inhibits the sulphation of  
15 glycosaminoglycans. The inhibition studies point to a critical role of these ECM compounds in tubulogenesis. Numerous studies have shown that branching morphogenesis of the ureter as well as branching of other epithelia requires an intact ECM. Since presence of chlorate after  
20 24 h does not influence tubulogenesis, GAGs do not seem to be involved in tubule maturation and differentiation. Tubule induction does, however, depend on sulfated GAGs in the first 24 h, the period essential for complete induction by the spinal cord. GAGs may act as co-  
25 receptors, facilitating presentation or increasing the local concentration of the ligand.

#### Wnt-4 signaling as a trigger for tubulogenesis

In order to test whether Wnt-4 expressing cells can rescue a Wnt-4 mutant mesenchyme, direct  
30 recombination experiments were carried out in culture. Induction of tubulogenesis in wild-type and Wnt-4 mutant metanephric mesenchyme by NIH3T3 cells stably expressing Wnt-4 was evaluated as follows. Isolated metanephric mesenchyme was placed on top of NIH3T3 cells expressing  
35 Wnt-4 which were supported by a nucleopore filter. After

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48 h and 96 h, cultures were monitored as whole mounts using bright field microscopy; after 144 h, the cultures were monitored as histological sections. Induction of tubulogenesis in wild-type and Wnt-4/Wnt-4 mutant

- 5 metanephric mesenchyme by Wnt-4 expressing cells were indistinguishable. Wnt-4-expressing cells were equally efficient at inducing tubule formation in wild type or Wnt-4 mutant metanephric mesenchyme (Table 5).

- Brightfield microscopy and histological analysis  
10 of specimen After 6 d in culture revealed the full spectrum of tubular differentiation including glomerulus formation.

- As with spinal cord mediated induction, Wnt-4 expression in the mesenchyme itself is not required for tubule  
15 formation, but supplying Wnt-4 in adjacent cells is sufficient to trigger the inductive process. These results suggest that whereas Wnt-4 plays an essential role in initial tubulogenesis, it may not be required for later morphogenesis of the tubule. As shown in Table 5,  
20 Wnt-1 expressing cells were also sufficient to trigger tubulogenesis in mesenchyme mutant for Wnt-4.

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Table 5: Induction of tubulogenesis in Wnt-4/Wnt-4 mutant metanephric mesenchyme by NIH3T3 cells expressing Wnt-4 or Wnt-1

#Exp	# Recombinants	#induced/#total		
5		+/+	Wnt-4/+	Wnt-4/Wnt-4
with NIH3T3 cells expressing Wnt-4:				
4	42	7/7	18/18	17/17
10				
with NIH3T3 cells expressing Wnt-1:				
2	20	5/5	11/12	3/3

Mammalian kidney development

Metanephric development is a highly coordinated process characterized by a continuous interaction of the epithelial ureter and the surrounding metanephric mesenchyme. Classical organ culture experiments have pointed to the fact that these two compartments achieve coordinated development by use of reciprocal signaling systems. First, the metanephric blastema induces a bud from the adjacent nephric duct which invades and branches into the mesenchyme. This process appears to be mediated by GDNF which is secreted by the metanephric mesenchyme and sensed by the c-ret/GDNFRa receptor complex on the ureter. Next, the metanephric mesenchyme undergoes tubulogenesis upon a permissive stimulus from the ureter.

Signals required for induction of tubulogenesis

In addition to Wnt-4, other Wnts may replace Wnt-4 activity in the mesenchyme. Using cell lines expressing various Wnt genes, Wnt-1, Wnt-3a, Wnt-7a, Wnt-7b, were

- 40 -

shown to evoke tubulogenesis in isolated metanephric mesenchyme. The results described herein suggest a different interpretation of the use of kidney cultures to elucidate the nature of the ureteric signal involved in inducing the mesenchyme. Experiments which have used heterologous sources of tubule inducers, e.g., the spinal cord, may not have been investigating the nature of ureteric signaling, but rather the mesenchymal action of signals such as Wnt-4. At present, the exact nature of ureteric signaling remains obscure. A primary signal might be required for a sufficient length of time to allow auto-induction of the mesenchyme by Wnt-4. Alternatively, a secondary signal from the ureter tip might be necessary to induce Wnt-4 expression in aggregating mesenchyme. In contrast to earlier studies, the data presented herein indicate that Wnt-11 does not play a role as a ureteric signal for mesenchymal aggregation. In the present studies, tubulogenesis was not detected with cells expressing Wnt-11.

Wnt-4 is a mesenchymal signal for tubulogenesis

Analysis of Wnt-4 mutants has demonstrated a critical role for Wnt-4 in kidney development. Homozygous pups die 24 h after birth due to small agenic kidneys consisting of undifferentiated mesenchyme intermingled with collecting duct tissue. Histological and marker analysis revealed that primary condensation of mesenchymal cells around the ureter tips as well as ureteric branching occurs normally. However, mutant kidneys quickly become growth retarded and the mesenchyme remains undifferentiated lacking pretubular cell aggregates and epithelial tubules. Since kidney size as well as cell death initially remain unaffected, proliferation is unlikely to be controlled by Wnt-4. Rather, the lack of Wnt-4 expression itself and of epithelial structures in the mutant mesenchyme indicates

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that Wnt-4 may autoinduce the epithelialization of condensed mesenchyme. Mesenchymally-derived Wnt-4 is not only required but also sufficient for induction of tubulogenesis in the mammalian kidney. Judging by  
5 histological and molecular markers, Wnt-4 can elicit the complete program of tubular differentiation in isolated metanephric mesenchyme. The activity of Wnt-4 contrasts with other factors thought to regulate mesenchymal development. For example, basic fibroblast growth factor  
10 (FGF) and epidermal growth factor (EGF) can both support mesenchymal survival but are not sufficient for tubulogenesis. Like Wnt-4, BMP-7 has been suggested to induce tubules, but loss-of-function studies indicate it is not essential for tubule formation in vivo as some  
15 glomeruli form in BMP7 mutants. In contrast, loss of Wnt-4 led to a complete absence of glomeruli.

Wnt-4 activity shows all the characteristics which have previously been ascribed to induction by dorsal spinal cord tissue. Signaling is cell-contact dependent.  
20 Below a certain pore size in the transfilter assay the formation of cellular processes which penetrate the filter pores is inhibited and isolated mesenchyme degenerates. Cell contact is required for induction of tubulogenesis, and Wnt proteins may interact with  
25 extracellular matrix (ECM) components.

Wnt-4 expression in the metanephric mesenchyme is initiated in the aggregating mesenchyme and maintained in the comma shaped bodies before it is downregulated in S-shaped bodies. Therefore, Wnt-4 likely has a later  
30 function in tubulogenesis.

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Table 15: Human Wnt-4-encoding nucleic acid

1 TGCAAGTGTC ACGGGGTGTC AGGCTCCTGT GAGGTAAAGA CGTGCTGGCG  
51 AGCCGTGCCG CCCTTCGCGC AGGTGGGTCA CGCACTGAAG GAGAAGTTTG  
101 ATGGTGCCAC TGAGGTGGAG CCACGCCGCG TGGGCTCCTC CAGGGCACTG  
151 GTGCCACGCA ACGCACAGTT CAAGCCGCAC ACAGATGAGG ACCTGGTGTA  
201 CTTGGAGCCT AGCCCCGACT TCTGTGAGCA GGACATGCGC AGCGGCGTGC  
251 TGGGCACGAG GGGCCGCACA TGCAACAAGA CGTCCAAGGC CATCGACGGC  
301 TGTGAGCTGC TGTGCTGTGG CCGCGGCTTC CACACGCCGC AGGTGGAGCT  
351 GGCTGAACGC TGCAGCTGCA AATTCCACTG GTGCTTGTTT TTGASTCGAC

SEQ ID NO: 10

Table 16: Human Wnt-7a-encoding nucleic acid

1 TGTAAGTGTC ACGGCGTGTC AGGCTCGTGC ACCACCAAGA CGTGCTGGAC  
51 CACACTGCCA CAGTTTCGGG AGCTGGGCTA CGTGCTCAAG GACAAGTACA  
101 ACGAGGCCGT TCACGTGGAG CCTGTGCGTG CCAGCCGCAA CAAGCGGCCC  
151 ACCTTCCTGA AGATCAAGAA GCCACTGTCTG TACCGCAAGC CCATGGACAC  
201 GGACCTGGTG TACATCGAGA AGTCGCCCAA CTACTGCGAG GGGGACCCGG  
251 TGACCGGCAG TGTGGGCACC CAGGGCCGCG CCTGCAACAA GACGGCTCCC  
301 CAGGCCAGCG GCTGTGACCT CATGTGCTGT GGGCGTGGCT ACAACACCCA  
351 CCAGTACGCC CGCGTGTGGC AGTGCAATTG TAAGTTCCAT TGGTGC

SEQ ID NO: 11

Table 17: Human Wnt-7b-encoding nucleic acid

```
1  GTAAAATGTC ACGGCGTGTC TGGCTCCTGC ACCACCAAAA CCTGCTGGAC
51  CACGCTGCCC AAGTTCCGAG AGGTGGGCCA CCTGCTGAAG GAGAAGTACA
101 ACGCGGCCGT GCAGGTGGAG GTGGTGCGGG CCAGCCGTCT GCGGCAGCCC
151 ACCTTCCTGC GCATCAAACA GCTGCGCAGC TATCAGAAGC CCATGGAGAC
201 AGACCTGGTG TACATTGAGA AGTCGCCCAA CTACTGCGAG GAGGACGCGG
251 CCACGGGCAG CGTGGGCACG CAGGGCCGTC TCTGCAACCG CACGTCGCCC
301 GGCGCGGACG GCTGTGACAC CATGTGCTGC GGCCGAGGCT ACAACACCCA
351 CCAGTACACC AAGGTGTGGC AGTGCAACTG CAAATTCCAC TGGTGCTGCT
401 CTAG
```

SEQ ID NO: 12

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It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

What is claimed is:

- 45 -

1. A method of stimulating kidney tubule formation in a post-natal mammal, comprising administering to said mammal a substantially pure Wnt polypeptide or a Wnt agonist, wherein said Wnt polypeptide is not Wnt-11.  
5
2. The method of claim 1, wherein said mammal is characterized as suffering from a kidney disorder.
3. The method of claim 1, wherein said mammal is an adult mammal.
- 10 4. The method of claim 2, wherein said disorder is chronic renal failure.
5. The method of claim 2, wherein said disorder is renal cell carcinoma.
5. The method of claim 2, wherein said disorder  
15 is polycystic kidney disease.
6. The method of claim 2, wherein said disorder is chronic obstructive uropathy.
7. The method of claim 2, wherein said disorder is virus-induced nephropathy.
- 20 8. The method of claim 7, wherein said virus is HIV-1.
9. The method of claim 1, wherein said Wnt polypeptide is a Wnt-1 class polypeptide.

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10. The method of claim 1, wherein said Wnt polypeptide is selected from the group consisting of Wnt-3a, Wnt-4, Wnt-7a, and Wnt-7b.

11. The method of claim 1, wherein said Wnt  
5 polypeptide is Wnt-4.

12. The method of claim 1, wherein said Wnt agonist is HLDAT86.

13. The method of claim 1, further comprising administering a sulfated glycosaminoglycan.

10 14. The method of claim 1, wherein said Wnt polypeptide or Wnt agonist is administered locally to a renal tissue.

15 15. The method of claim 14, wherein said Wnt polypeptide or Wnt agonist administered by retrograde perfusion of said renal tissue.

16. The method of claim 1, wherein said Wnt polypeptide or Wnt agonist is administered ex vivo to an explanted renal tissue.

20 17. The method of claim 1, wherein said Wnt agonist is a peptide mimetic.

18. The method of claim 1, wherein said Wnt polypeptide has an amino acid sequence at least 85% identical to SEQ ID NO:1, 2, 3, 4, or 5, and wherein said Wnt polypeptide induces tubulogenesis.

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19. A method of stimulating kidney tubule formation in a post-natal mammal, comprising administering to said mammal a substantially pure nucleic acid encoding a Wnt polypeptide or a Wnt agonist.

5 20. An *ex vivo* mammalian kidney comprising an substantially pure exogenous Wnt polypeptide.

PCT

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>C07K 14/475, A61K 48/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 00/61630</b> <b>(43) International Publication Date:</b> 19 October 2000 (19.10.00)
<b>(21) International Application Number:</b> PCT/US99/07745 <b>(22) International Filing Date:</b> 8 April 1999 (08.04.99)  <b>(71) Applicant (for all designated States except US):</b> PRESIDENT AND FELLOWS OF HARVARD COLLEGE [US/US]; 17 Quincy Street, Cambridge, MA 02138 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> MCMAHON, Andrew, P. [GB/US]; 128 Kendall Road, Lexington, MA 02173 (US). KISPERT, Andreas [DE/DE]; Lindenweg 12, D-74838 Limbach (DE). VAINIO, Seppo [FI/FI]; Tohtorintie 6, FIN-90570 Oulu (FI).  <b>(74) Agent:</b> BEATTIE, Ingrid, A.; Mintz, Levin, Cohn, Ferris, Glovsky & Popeo PC, One Financial Center, Boston, MA 02111 (US).		<b>(81) Designated States:</b> CA, JP, US. European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> With international search report.
<b>(54) Title:</b> INDUCTION OF KIDNEY TUBULE FORMATION  <b>(57) Abstract</b>  The invention provides a method of stimulating kidney tubule formation in a post-natal mammal by administering to the mammal a substantially pure Wnt polypeptide or Wnt agonist.		

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the specification of which:

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100  
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TRA 1624973v1

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Andreas Dispe  
Inventor's Signature

27. March 2002  
Date

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Boston, Massachusetts 02111

$$\frac{1}{\Gamma(\alpha)} \int_0^t (t-s)^{\alpha-1} f(s) ds = \frac{1}{\Gamma(\alpha)} \int_0^t (t-s)^{\alpha-1} f(s) ds + \frac{1}{\Gamma(\alpha)} \int_t^\infty (t-s)^{\alpha-1} f(s) ds$$

Post Office Address: 128 Kendall Road, Lexington, MA 02173

Post Office Address: Lindenweg 12, D-74838, Limbach, Germany

Post Office Address: Tohtorintie 6, FIN-900570 Oulu, Finland

- 3 -

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## <120> Induction of Kidney Tubule Formation

<140> 09/937,735

<141> 2001-09-28

<150> PCT/US99/07745

<151> 1999-04-08

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&lt;170&gt; PatentIn Ver. 2.1

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